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Claims for the following Contracting State: ES.

(54) Human platelet-derived growth factor receptor.

57) A DNA sequence encoding the human platelet-derived growth factor receptor (hPDGF-R) has now been isolated and sequenced. An expression construct comprises the sequence encodes a receptor that can be secreted or incorporated into the membrane of a mammalian cell. The incorporated receptor is functionally equivalent to the wild-type receptor, conferring a PDGF-sensitive mitogenic response on cells lacking the receptor. The construct can be used for enhancing PDGF response of cells, determining the regions involved in transducing the signal in response to PDGF binding, providing mutated analogs and evaluating drugs for their physiologic activity.

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Description

HUMAN PLATELET-DERIVED GROWTH FACTOR RECEPTOR

The present invention relates to growth factors and their receptors and, in particular, to human platelet-derived growth factor receptor.

5 Platelet-derived growth factor (PDGF) is a major mitogen for cells of mesenchymal origin. The protein is a 32 kDa protein heterodimer composed of two polypeptide chains, A and B, linked by disulfide bonds. In addition to the PDGF AB heterodimer, two homodimeric forms of PDGF, denoted AA and BB, have been identified. At the present time, there is no direct proof that the AA form of PDGF can bind to PDGF receptors.

10 The first event in PDGF-mediated mitogenesis is the binding of PDGF to its receptor at the cell membrane. This interaction triggers a diverse group of early cellular responses including activation of receptor tyrosine kinase, increased phosphatidylinositol turnover, enhanced expression of a group of genes, activation of phospholipase A2, changes in cell shape, increase in cellular calcium concentration, changes in intracellular pH, and internalization and degradation of bound PDGF. These changes are followed by an increase in the rate of proliferation of the target cells.

15 While the ability of a polypeptide to stimulate growth of a particular cell type *in vitro* does not prove that it serves the same function *in vivo*, the role of many growth factors on cells is being studied to attempt to determine the role that the factors play in the whole organism. *In vitro*, platelet-derived growth factor is a major polypeptide mitogen in serum for cells of mesenchymal origin such as fibroblasts, smooth muscle cells and glial cells. *In vivo*, PDGF circulates stored in the α granules of blood platelets and does not circulate freely in blood. During blood clotting and platelet adhesion, the granules are released, often at sites of injured blood vessels implicating PDGF in the repair of blood vessels. PDGF also stimulates migration of arterial smooth muscle cells from the medial to the intimal layer of the artery where they then proliferate as an early response to injury.

25 PDGF is being studied to determine how cell proliferation is controlled in the body. The growth factor has been implicated in wound healing, in atherosclerosis, and in stimulating genes associated with cancerous transformation of cells, particularly *c-myc* and *c-fos*. Therefore, PDGF agonists may be useful in promoting wound healing. PDGF antagonists may be useful in preventing atherosclerosis, in retarding blood vessel narrowing that occurs after cardiovascular intervention and in controlling cancerous proliferation.

30 The mouse PDGF receptor has been identified, purified (Daniel et al., Proc. Natl. Acad. Sci. USA (1985) 82:2684-2687), and sequenced (Yarden et al., Nature (1986) 323:226-232).

35 A DNA sequence encoding the human platelet-derived growth factor receptor (hPDGF-R) has now been isolated and sequenced. An expression construct comprising the sequence encodes a receptor that can be secreted or incorporated into the membrane of a mammalian cell. The incorporated receptor is functionally equivalent to the wild-type receptor, conferring a PDGF-sensitive mitogenic response on cells lacking the receptor. The construct can be used for enhancing PDGF response of cells, determining the regions involved in transducing the signal in response to PDGF binding, providing mutated analogs and evaluating drugs for their physiologic activity.

40 DESCRIPTION OF SPECIFIC EMBODIMENTS

Methods for producing human platelet-derived growth factor (hPDGF-R) and nucleic acid constructs for such production are provided as well as cells comprising the hPDGF-R where the composition and cells may find use in diagnosis, evaluation of drugs affecting the transduction of the hPDGF-R signal and in the treatment of diseases associated with hPDGF-R. Particularly, an expression construct encoding hPDGF-R is provided. The construct can be used to transfect cells providing a membrane-bound receptor that is functionally equivalent to the wild-type receptor, and conferring a PDGF-sensitive mitogenic response on cells lacking the receptor. The transfected cells can be used as a model for studying the PDGF-induced response of cells, determining the regions involved in transducing the signal in response to PDGF binding and evaluating drugs for their physiologic activity. The encoded receptor or its binding region also find use in evaluating PDGF agonists. Other utilities for the DNA sequence include use of fragments of the sequence as probes to detect deletions in the region of chromosome 5 where a number of growth-control related genes are clustered.

55 The nucleotide sequence of a cDNA sequence encoding hPDGF-R is set forth in Table 1 together with the deduced amino acid sequence of the receptor precursor. The sequence beginning at the amino acid numbered 1 corresponds to the amino terminus of human PDGF-R. The first 32 amino acids (designated -32 to -1) encode the signal peptide sequence. The dark bar underlines the transmembrane sequence (amino acid residues 500 to 524). Potential N-glycosylation sites are indicated by a line. The polyadenylation site in the 3' end of the cDNA has been underlined.

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The DNA compositions of this invention may be derived from genomic DNA or cDNA, prepared by synthesis or combinations thereof. The DNA compositions may include the complete coding region encoding hPDGF-R or fragments thereof of interest, usually comprising at least 8 codons (24 bp), more usually at least 12 codons, where one or more introns may be present. While for the most part the wild-type sequence will be employed, in some situations one or more mutations may be introduced, such as deletions, substitutions or insertions resulting in changes in the amino acid sequence or providing silent mutations. The genomic sequence will usually not exceed 50 kbp, more usually not exceed about 10 kbp, preferably not greater than 6 kbp.

The DNA fragment encoding hPDGF-R finds use to isolate DNA encoding PDGF receptors of other species which share substantial homologies with hPDGF-R. Portions of the DNA fragment having at least about 10 nucleotides, usually at least about 20 nucleotides, and fewer than about 6 knt (kilonucleotides), usually fewer than about 0.5 knt, from a DNA sequence encoding hPDGF-R find use as probes. The probes can be used to determine whether mRNA encoding hPDGF-R is present in a cell.

Additionally, the human PDGF receptor gene is located at a site on chromosome 5 where a number of growth control related genes are clustered. At least one genetic disease, 5q minus syndrome, has been shown to involve a deletion in this region. Fragments of the hPDGF-R gene sequence may be used as a marker to probe the structure of this important region of the genome and to diagnose genetic diseases associated with this area of the genome.

The DNA fragment or portions thereof can also be used to prepare an expression construct for hPDGF-R. The construct comprises a DNA sequence encoding hPDGF-R under the transcriptional control of the native or other than the native promoter. Usually the promoter will be a eukaryotic promoter for expression in a mammalian cell, where the mammalian cell may or may not lack PDGF receptors. In cases where one wishes to expand the DNA sequence or produce the receptor protein or fragments thereof in a prokaryotic host, the promoter may also be a prokaryotic promoter. Usually a strong promoter will be employed to provide for high level transcription and expression.

The expression construct may be part of a vector capable of stable extrachromosomal maintenance in an appropriate cellular host or may be integrated into host genomes. The expression cassette may be bordered by sequences which allow for insertion into a host, such as transposon sequences, lysogenic viral sequences, or the like. Normally, markers are provided with the expression cassette which allow for selection of host containing the expression cassette. The marker may be on the same or a different DNA molecule, desirably the same DNA molecule.

In mammalian cells, the receptor gene itself may provide a convenient marker. However, in prokaryotic cells, markers such as resistance to a cytotoxic agent, complementation of a auxotrophic host to prototrophy, production of a detectable product, etc. will be more convenient.

The expression construct can be joined to a replication system recognized by the intended host cell. Various replication systems include viral replication systems such as retroviruses, simian virus, bovine papilloma virus, or the like. In addition, the construct may be joined to an amplifiable gene, e.g., DHFR gene, so that multiple copies of the PDGF-R gene may be made.

Introduction of the construct into the host will vary depending upon the particular construction. Introduction can be achieved by any convenient means, including fusion, conjugation, transfection, transduction, electroporation, injection, or the like, as amply described in the scientific literature. The host cells will normally be immortalized cells, that is cells that can be continuously passaged in culture. For the most part, these cells may be convenient mammalian cell lines which are able to express PDGF-R and where desirable, process the polypeptide so as to provide a mature polypeptide. By processing is intended glycosylation, ubiquitination, disulfide bond formation, or the like. Usually the host will be able to recognize the signal sequence for inserting hPDGF-R into the membrane of the cell. If secretion is desired, the transmembrane locator sequence may be deleted or mutated to prevent membrane insertion of the protein.

A wide variety of hosts may be employed for expression of the peptides, both prokaryotic and eukaryotic. Useful hosts include bacteria, such as *E. coli*, yeast, filamentous fungus, immortalized mammalian cells, such as various mouse lines, monkey lines, Chinese hamster ovary lines, human lines, or the like. For the most part, the mammalian cells will be immortalized cell lines. In some cases, the cells may be isolated from a neoplastic host, or wild-type cells may be transformed with oncogenes, tumor causing viruses, or the like.

Under many circumstances, it will be desirable to transfect mammalian cells which lack a PDGF receptor where the signal sequence directs the peptide into the cell membrane. Lymphocytes and cardiac myocytes are primary cells which lack a receptor. Also, Chinese hamster ovary cells (CHO), epithelial cells lines and a number of human tumor cell lines lack PDGF receptors.

Transfected cells find use as a model for studying cellular responses to PDGF. For controlled investigation, mammalian cells which lack a PDGF receptor can be transfected with an expression construct comprising a DNA sequence encoding hPDGF-R. Cells are produced that encode a receptor that is functionally equivalent to the wild-type receptor and confer an PDGF-sensitive mitogenic response on the cell. In this way, the binding properties of the naturally-occurring PDGF may be analyzed, fragments tested as well as synthetic compounds both proteinaceous and non-proteinaceous. As demonstrated in the Experimental section, transfected cells were used to determine that the AA form of PDGF activates the receptor tyrosine kinase.

In addition to studying PDGF-mediated mitogenesis, the transfected cells can be used to evaluate a drug's ability to function as a PDGF agonist or antagonist. In particular, transfected cells can be contacted with the test drug, and the amount of receptor tyrosine kinase activation or the rate of DNA synthesis can be

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control cells
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determined in comparison to control cells in the presence or absence of PDGF, or analogs thereof of known activity.

The hPDGF-R protein expressed by transfected cells also finds use. If the peptide is secreted, the peptide may be isolated from the supernatant in which the expression host is grown. If not secreted, the peptide may be isolated from a lysate of the expression host. The peptide may then be isolated by convenient techniques employing HPLC, electrophoresis, gradient centrifugation, affinity chromatography, particularly using PDGF, etc., to provide a substantially pure product, particularly free of cell components and contaminants.

The receptor protein or amino acids beginning at about 33 through about 500 of the amino terminal sequence of the receptor which form the external domain, binding portion of the receptor protein find use to affinity purify PDGF. The external domain can also be used affixed to a solid substrate or free in solution to determine drugs useful as PDGF agonists and antagonists.

The protein or the intracellular portion of the protein, beginning at about amino acid 525 through the carboxy terminal amino acid of hPDGF-R, also find use as an enzyme having tyrosine kinase activity. Additionally, amino acids 1 through 32 of the amino terminal sequence of the receptor comprise a signal sequence which directs the structural protein through the membrane of a transfected cell. The signal sequence can be used with hPDGF-R, but also finds use with other proteins.

Peptides or portions thereof may also be used for producing antibodies, either polyclonal or monoclonal. Antibodies are produced by immunizing an appropriate vertebrate host, e.g. mouse, with the peptide by itself, or in conjunction with a conventional adjuvant. Usually two or more immunizations will be involved, and the blood or spleen will be harvested a few days after the last injection.

For polyclonal antisera, the immunoglobulins may be precipitated, isolated and purified, including affinity purification. For monoclonal antibodies, the splenocytes normally will be fused with an immortalized lymphocyte, e.g. a myeloid line, under selective conditions for hybridomas. The hybridomas may then be cloned under limited dilution conditions and their supernatants screened for antibodies having the desired specificity. Techniques for producing antibodies are well known in the literature and are exemplified by U.S. Patent Nos. 4,381,292, 4,451,570 and 4,618,577.

EXPERIMENTAL

Screening of Human Kidney λ GT11 cDNA Library and Human Placenta λ GT10 cDNA Library

A full-length DNA sequence encoding the mouse PDGF receptor (mPDGF-R) protein was used as a probe to screen 250,000 plaques of a human kidney cDNA library. Nick translation was used to prepare a probe with specific activity of 12×10^8 cpm per μ g. The filters were incubated with the probe (10^5 cpm per ml) in hybridization buffer containing 30% formamide, 1X Denhardt's solution, 5X SSC, 0.02M sodium phosphate pH 6.5 and 500 μ g per ml of salmon sperm DNA. After 14 hr. of hybridization at 40°C, the filters were washed four times at 55°C with 0.2X SSC and 0.1% SDS and two additional times at 65°C with 0.2X SSC. The filters were then air dried and exposed for 16 hrs.

Ten positive clones were obtained which were rescreened with the full-length mPDGF-R probe. Individual clones were isolated and analyzed by restriction analysis using EcoRI endonuclease. The clone containing the largest insert (2.3 kb), designated clone HK-6, was further characterized and sequenced using dideoxy terminators. Clone HK-6 contained the receptor sequence from nucleotide 3554 to nucleotide 5691 plus nine bases from the poly A tail.

A nick-translated probe, prepared from the 2.3 kb HK-6 DNA, was used to screen 250,000 plaques of a human placenta cDNA library. This screening was performed at high hybridization stringency (50% formamide in the hybridization buffer described above). The filters were incubated with 5×10^5 cpm per ml of probe for 14-16 hrs. at 42°C. The filters were then washed at 65°C in 0.1% SSC and 0.1% SDS four times.

After secondary screening with the HK-6 probe, seven clones were selected and analyzed by restriction digestion with EcoRI endonuclease. A clone (HP-7) that contained a 4.5 kb insert was selected and characterized. The sequence of that clone is described in Table 1.

Construction of Expression Vector

The 4.5 kb DNA fragment containing the complete coding sequence for the human PDGF receptor was isolated from the HP-7 clone EcoRI digestion. The gel purified fragment was cloned into the EcoRI site in the polylinker region of SV40 expression vector PSV7C. The pSV7d expression vector (provided by P. Luciw, University of California, Davis) was a pML derivative containing the SV40 early promoter region (SV40 nucleotides 5190-5270), a synthetic polylinker with restriction sites for EcoRI, SmaI, XbaI, and SalI followed by three translation terminator codons (TAA) and the SV40 polyadenylation signal (SV40 nucleotides 2556-2770) (Truett et al., DNA (1984) 4333-349). The EcoRI fragment containing the cDNA sequence obtained from the HP-7 clone was inserted at the EcoRI site of the pSV7d. In the resulting expression vector, the hPDGF-receptor gene was under transcriptional control of the SV40 promoter.

To ensure the proper orientation of the PDGF receptor insert (4.5 kb) with respect to the SV40 promoter, the positive clones were digested with SmaI end nuclease which cuts at position 573 of the receptor sequence and in the polylinker region of the expression vector.

Clones containing the receptor in the proper transcriptional orientation released a 4.0 kb insert in addition to the 3.2 kb fragment containing the expression vector plus 573 base pairs of the 5' end of the receptor. This plasmid, PSVRH5 was used to co-transfect cells with PSV2 neo plasmid that confers resistance to the antibiotic neomycin.

Cell Culture and Transfection of CHO Cells

CHO cell clone KI, obtained from the U.C.S.F. Tissue Culture Facility, were grown in Ham's F-12 media supplemented with 10% FCS (U.C.S.F. Tissue Culture Facility) and penicillin and streptomycin at 37°C in 5% CO₂/95% air.

pSVRH5 plasmid DNA (10 µg) and pSV2 neo (1 µg) were used to co-transfect 1 X 10⁶ CHO cells by the calcium precipitation technique (Van der Eb et al., *Methods Enzymology* (1980) 65:826-839), with the addition of 10 µg chloroquine diphosphate (CDP) to prevent degradation of the transfected DNA (Luthman and Magnusson, *Nucl. Acid Res.* (1983) 11:1295-1308). After 12 hrs. of exposure to the DNA, the cells were trypsinized and replated at 1:5 dilution. Twenty-four hours later, the antibiotic G418 (GIBCO), an analog of neomycin, was added to the cultures at a concentration of 400 µg/ml.

After two weeks under selection, independent colonies were picked and transferred to 24-well plates. Confluent cultures were assayed for the presence of PDGF receptor by immunoblot using anti-receptor antibodies. Colonies that were positive by this assay were single-cell cloned by end-limiting dilution.

Stable transfected clones were tested for the expression of the PDGF receptor message measured by RNA protection assays (Zinn et al., *Cell* (1983) 34:865-879) and for the presence of PDGF-stimulated receptor protein detected by antiphosphotyrosine antibodies (Frackelton et al., *J. Biol. Chem.* (1984) 259:7909-7915).

Expression of hPDGF-R cDNA in CHO cells

CHO cells transfected with plasmid DNA containing the human receptor cDNA under the transcriptional control of the SV40 early promoter (CHO-HR5) and CHO cells transfected with a similar plasmid containing the mouse receptor cDNA (CHO-R18) were solubilized as previously described (Escobedo et al., *J. Biol. Chem.* (1988) 263:1482-1487). Extracts were analyzed by Western blot analysis using an antibody that specifically recognizes sequences in the receptor carboxy-terminal region as previously described (Escobedo et al., *supra*; Keating et al., *ibid.* (1987) 262:7932-7937). The 195 kDa protein is the mature receptor and the 160 kDa protein is the receptor precursor.

The expression of the receptor protein in the transfectants was demonstrated by using antibodies that recognize an intracellular sequence in the receptor. The clone that had the highest level of human receptor expression was chosen for further study. This transfectant had receptors that were labeled with ¹²⁵I-PDGF as shown by the competitive binding studies described below.

Competitive Binding of the Different Forms of PDGF to its Receptor

The ability of the human recombinant AA and BB homodimers (Collins et al., *Nature* (1987) 328:621-624) to compete for the receptor sites and displace ¹²⁵I-labeled PDGF was studied. Each homodimer was produced selectively by a yeast expression system (Brake et al., *Proc. Natl. Acad. Sci. (USA)* (1984) 81:4642-4646) and was purified from yeast media that is devoid of other mesenchymal cell growth factors, thus avoiding the artifact of contamination by factors that might be present in mammalian expression systems.

BALB/c 3T3 cells and CHO transfectants (CHO-HR5) were incubated with ¹²⁵I-PDGF (William et al., *ibid.* (1982) 79:5067-5070) in the presence of increasing concentrations of AA and BB. Binding was carried out at 37°C for 45 min. In whole cell suspension. Unbound, radiolabeled PDGF was removed by centrifugation on a Ficoll gradient (Orchansky et al., *J. Immunol.* (1986) 136:169-173). Non-specific binding, determined by incubating CHO cells with ¹²⁵I-PDGF, accounted for 25 percent of the bound radioactivity.

The binding study demonstrated that the transfected cells can be used as a model to study the interaction of hPDGF with its receptor. In particular, this study demonstrated that the transfected human receptor was functionally identical to the native mouse receptor as indicated by the following results. Both AA and BB forms of PDGF competed for the ¹²⁵I-PDGF labeled sites in the human receptor transfectants. For the transfected human receptor as well as the native mouse receptor, the BB form was of higher affinity than the AA form. When expressed in yeast, the AA form of PDGF may be processed aberrantly, giving it a lower affinity than the BB form for both the transfected cells and mouse 3T3 cells. The consistency of the pattern of competition shows that the AA form interacts with the transfected human receptor in the same way as it does with the native mouse receptor and demonstrates that these receptors are functionally identical.

Activation of the PDGF Receptor Tyrosine Kinase

The ability of recombinant AA and BB homodimers and of human partially purified AB PDGF to activate the receptor tyrosine kinase was studied. The yeast-derived AA and BB homodimeric forms and the platelet-derived AB form stimulated autophosphorylation of the transfected human receptor.

BALB/c 3T3 cells and CHO cells transfected with the human PDGF receptor cDNA (CHO-HR5) were incubated with increasing amounts of different forms of PDGF (AA, BB and AB). Following polyacrylamide-SDS electrophoresis, the phosphorylated receptor was identified by Western blot using an antiphosphotyrosine antibody (Wand, *Mol. Cell. Biol.* (1985) 5:3640-3643).

The receptor protein co-migrated with the 200 kDa molecular weight marker. The concentration of each form

the was effective in stimulating autophosphorylation of the transfected human receptor was identical to the concentration that gave a similar autophosphorylation to the native mouse 3T3 receptor or the transfected mouse receptor.

These results show for the first time that the AA form of PDGF activates the receptor tyrosine kinase. Prior to use of the transfected cells, there was no demonstration that the AA form had hPDGF activity or that a single receptor was capable of recognizing all three forms of PDGF. Further, the results demonstrate that the human cDNA encodes a receptor that is functionally equivalent to the wild-type receptor that is responsible for PDGF-stimulated tyrosine kinase activity in mouse 3T3 cells.

Thus, the transfected cells are useful models for studying PDGF-induced mitogenic responses.

Rate of DNA Synthesis in CHO Transfected Cells

BALB/c 3T3 cells and CHO cells transfected with human PDGF receptor cDNA (CHO-HR5) were incubated with saturating concentrations of the three forms of PDGF. Untreated cells and cells treated with fetal calf serum (FCS) were used as negative and positive controls, respectively. The level of ³H-thymidine incorporation into DNA was determined by measuring the radioactivity of the acid-precipitable material as previously described (Escobedo, supra).

Transfection of CHO cells with either human or mouse PDGF receptor conferred a PDGF-sensitive mitogenic response. All forms of PDGF stimulated DNA synthesis in both the human receptor transfectant and the mouse cells bearing the native receptor.

These data showed that the A chain homodimer and the B chain homodimer, like the AB platelet-derived form, were mitogens that can act through the receptor encoded by this human cDNA sequence. The mitogenic action of these forms of PDGF on mouse 3T3 cells and CHO cells containing the transfected human receptor demonstrate that the responses were mediated by functionally identical receptors.

These studies were made possible by the availability of growth factor preparations devoid of contamination with other growth factors and by use of a receptor expression system in which all of the measured PDGF responses could be attributed to this single transfected receptor cDNA.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Claims

1. A DNA fragment of fewer than about 50 kbp encoding human platelet-derived growth factor receptor (hPDGF-R).

2. A DNA fragment as claimed in Claim 1 wherein the fragment comprises a cDNA sequence of less than about 6 kbp.

3. A probe comprising a sequence consisting essentially of at least about 10 nt of the DNA sequence encoding hPDGF-R.

4. A probe as claimed in Claim 3 wherein the probe has from about 25 nucleotides to 100 nucleotides.

5. An expression construct for hPDGF-R comprising in the 5'-3' direction of transcription, a promoter and under the transcriptional regulation of the promoter, a DNA sequence encoding hPDGF-R joined to other than DNA naturally joined to the hPDGF-R-encoding DNA sequence.

6. An expression construct as claimed in Claim 5 wherein the promoter is a eukaryotic promoter.

7. An hPDGF-R fragment having PDGF receptor binding activity consisting essentially of amino acids beginning at about 33 through about 500 of the amino-terminal sequence of hPDGF-R.

8. A substantially pure preparation of hPDGF-R or physiologically active fragments thereof.

9. A cell transfected by an expression construct for hPDGF-R comprising in the 5'-3' direction of transcription, a promoter and under the transcriptional regulation of the promoter, a DNA sequence encoding hPDGF-R joined to other than DNA naturally joined to said hPDGF-R-encoding DNA sequence.

10. A method of evaluating a drug's ability to function as a hPDGF agonist or antagonist comprising:

(a) contacting mammalian cells with the drug which mammalian cells comprise an hPDGF receptor as a result of transfecting said cells with an expression construct comprising a DNA sequence encoding hPDGF-R with the drug; and

(b) determining the amount of a PDGF-induced response in the cells in comparison to untransfected cells or a drug providing a known response.

Claims for the following Contracting State: ES

1. A process for the preparation of a DNA fragment of fewer than about 50 kbp encoding human platelet-derived growth factor receptor (hPDGF-R), the process comprising coupling successive nucleotides and/or ligating oligonucleotides.

claim 16 of 07/151,414

but this is not true of 67-6

applicant argued that there must be PDGF added

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2. A process as claimed in Claim 1 wherein the fragment comprises a cDNA sequence of less than about 6 kbp.

3. A process for the preparation of a probe comprising a sequence consisting essentially of at least about 10 nt of the DNA sequence encoding hPDGF-R, the process comprising coupling successive nucleotides and/or ligating oligonucleotides.

4. A process as claimed in Claim 3 wherein the probe has from about 25 nucleotides to 100 nucleotides.

5. A process for the preparation of an expression construct for hPDGF-R comprising in the 5'-3' direction of transcription, a promoter and under the transcriptional regulation of the promoter, a DNA sequence encoding hPDGF-R joined to other than DNA naturally joined to the hPDGF-R-encoding DNA sequence the process comprising coupling successive nucleotides and/or ligating oligonucleotides.

6. A process as claimed in Claim 5 wherein the promoter is a eukaryotic promoter.

7. A process for the preparation of an hPDGF-R fragment having PDGF receptor binding activity consisting essentially of amino acids beginning at about 33 through about 500 of the amino-terminal sequence of hPDGF-R, the process comprising coupling successive amino acid residues.

8. A process for the preparation of a substantially pure preparation of hPDGF-R or physiologically active fragments thereof, the process comprising coupling successive amino acid residues.

9. A process for the preparation of a transfected cell, the process comprising transfecting a cell by an expression construct for hPDGF-R comprising in the 5'-3' direction of transcription, a promoter and under the transcriptional regulation of the promoter, a DNA sequence encoding hPDGF-R joined to other than DNA naturally joined to said hPDGF-R-encoding DNA sequence.

10. A method of evaluating a drug's ability to function as a hPDGF agonist or antagonist comprising:

(a) contacting mammalian cells with the drug which mammalian cells comprise an hPDGF receptor as a result of transfecting said cells with an expression construct comprising a DNA sequence encoding hPDGF-R with the drug; and

(b) determining the amount of a PDGF-induced response in the cells in comparison to untransfected cells or a drug providing a known response.